

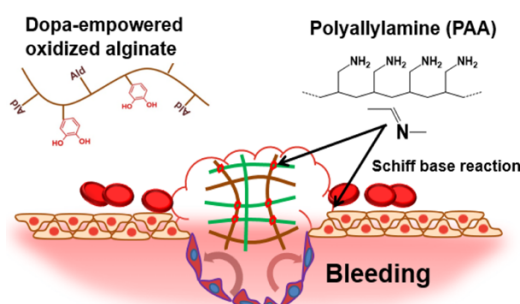
# Dopa-Empowered Schiff Base Forming Alginate Hydrogel Glue for Rapid Hemostatic Control

Chung Kil Song<sup>†</sup>  
Min-Kyoung Kim<sup>†</sup>  
Junghan Lee  
Enkhzaya Davaa  
Rengarajan Baskaran  
Su-Geun Yang\*

WCSL, Department of New Drug Development, Inha University College of Medicine, B-308, Chungsusuk Bldg, 366, Seohaе-Daero, Jung-Gu, Incheon 22332, Korea

Received March 8, 2018 / Revised June 30, 2018 / Accepted July 14, 2018

**Abstract:** In this study we prepared tissue-adhesive hemostatic glue and assessed hemostatic effects on hepatic bleeding animal model. Alginate was used as primary polymer for the fabrication of hemostatic glue, oxidized for the introduction of the tissue-adhesive Schiff base forming aldehyde and then encoded with mussel-inspired dopa. In addition, polyallylamine (PAA) was selected for as an intra-structuring polymer which ensures the gel strength and allows instantaneous glue formation on the bleeding spot. Primary glue (OA glue) was quickly formed within 5 to 10 seconds after the mixing oxidized alginate (OA) with PAA. The degree of oxidation and the mixing ratio of OA and PAA were precisely determined based on glue formation time and gel strength. And the extend of dopa conjugation on OA was determined by the tissue adhesion force and the elasticity of the glue (Dopa-OA glue). Especially, elasticity of Dopa-OA glue was significantly enhanced after introduction of dopa to OA. Functional assay of Dopa-OA glue on hepatic bleeding animal model showed much enhanced hemostatic action. Dopa-OA glue is expected to provide novel injectable tissue adhesives for the treatment of hemorrhage caused by clinical procedures or trauma.



**Keywords:** alginate, hemostatic glue, schiff base, dopa, tissue adhesive.

## 1. Introduction

Uncontrolled hemorrhage is responsible for over 40 percent of trauma-related deaths in the emergency care.<sup>1</sup> Surgical hemorrhage is also highly associated with mortality of hospital patients. Surgical fasteners such as sutures, staples, and wires are mainly used for the treatment of uncontrolled hemorrhage. But these treatments have serious drawbacks such as scarring and pain. In addition, they are not suitable for more complicated procedures such as stopping leaks of body fluids or air from blood vessels and tissues. Surgical adhesives have been developed as an adjunct or alternative to surgical fasteners. These adhesives offer many advantages because they can seal air leakages and eliminate the risk of needle-stick injury to the surgeon, as well as reduce surgery duration, tissue handling, and patient blood loss. They can also alleviate surgical complications such as infection. Surgical adhesives are easy to apply, produce a high-quality

and -strength seal, and do not need to be removed.<sup>2,3</sup> There are several types of commercially available tissue-adhesives, which are classified as natural, biological, synthetic, semi-synthetic and biomimetic. Synthetic adhesives are mostly made of cyanoacrylate because of their fast polymerization property and are therefore easy to use, waterproof and provide adequate mechanical properties.<sup>2</sup> Despite its merits, cyanoacrylate adhesives have major limitation in tissue toxicity. Tissue toxicity of cyanoacrylate results from the exothermic reaction of polymerization and the degradation of alkyl chains into cyanoacetate and formaldehyde, which cause inflammation and necrosis.<sup>4</sup>

Many researchers have attempted to modify the chemical structure of cyanoacrylate to reduce toxicity, but they have found cyanoacrylate adhesives are not suitable for internal bleeding control. Biological or natural adhesives are now becoming a potential alternative to synthetic adhesives. However, biological adhesives based on albumin or prothrombin have the potential to transmit viral infections or induce immune responses.<sup>5,6</sup> In addition, access to these adhesives is very limited by their high cost and inadequate application methods (*i.e.*, refrigerated storage and pre-mixing and curing before the application) in the operation room. On the other hand, natural adhesives have the advantage of not only excellent mechanical bonding properties but also biocompatibility without toxic side effects.

Polysaccharides have demonstrated clinical usefulness in variety of clinical applications and selected as a major component of natural tissue adhesives. Alginate is an anionic linear

**Acknowledgments:** This research was supported by Civil Military Co-Technology Development Program from Civil Military Technology Cooperation Center (16-CM-SS-07), and by Korea Research Fellowship Program and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2017R1A2A2A07001272, 2017R1A6A3A11035722, 2017R1D1A1B03035654, 2016H1D3A1938159 and 2018R1A6A1A03025523), and partly supported by WCSL (World Class Smart Lab) research grant directed by Inha University.

\*Corresponding Author: Su-Geun Yang (sugeun.yang@inha.ac.kr)

<sup>†</sup>Both authors contributed equally to this work

polysaccharide composed of (1-4)-linked  $\beta$ -D-mannuronate (M) and (1-4)-linked  $\alpha$ -L-guluronate (G) residues. Alginate has a high water-absorbing capacity and easily forms gel in the presence of divalent cations such as calcium. These properties of alginate are very attractive for the design of wound covering materials.<sup>7,8</sup> However calcium alginate hydrogel is inherently non-degradable in the body and does not display enough tissue adhesiveness for wound-covering or hemostatic action. In addition, high calcium content can inhibit cell growth and tissue recovery.<sup>9,10</sup> Many researchers have tried to make tissue adhesive alginate for clinical applications and found partially oxidized alginate (di-aldehyde derivatives) displays strong tissue-adhesive property.<sup>11-13</sup> Oxidation of the alginate makes the aldehyde form a Schiff-base with the amine residues of the tissue protein and a chemical structure that exerts tissue adhesion. However, the oxidized polysaccharides by itself do not have enough gel strength to control the high-pressure bleeding and require adjuvant polymer (*i.e.*, amine-rich polymers). Two polymers are mixed together before the application and form hemostatic adhesive glue at the bleeding site. The gel strength and elasticity of adhesive should be properly adjusted to achieve a reliable hemostatic action. The required physical properties can be controlled by adjusting the mixing ratio of polymers, the aldehyde content of alginate, and introduction of other functional groups to alginate. In this study, mussel-inspired dopamine was further introduced to Schiff-base forming oxidized alginate (OA). The application of mussel-inspired chemistry to tissue adhesives has been studied for decades and continues to attract the interest of scientists due to their functional efficacy and ease of chemical cross-linking.<sup>14-16</sup> Dopa is oxidized to a quinone form and reacts strongly with biomacromolecules as well as organic and inorganic materials *via* Schiff base reactions or Michael-type additions.<sup>14</sup> This property of dopa was employed to improve tissue-adhesiveness of Schiff-base forming oxidized alginate in this study.

In this study, we designed oxidized alginate hydrogel glue (OA glue; mixture of oxidized alginate and polyallylamine), and dopa-introduced oxidized alginate hydrogel glue (Dopa-OA glue). The aldehyde content of OA was precisely adjusted based on gelation time and gel strength. Mixing ratio of OA and PAA was also explored by rheological property of the acquired OA glues. And the introduction amount of dopa on alginate were examined by the tissue adhesive properties of the hydrogel glues. Hemostatic efficacy of glues was evaluated *in vivo* using a surgical hepatic bleeding mouse model.

## 2. Experimental

### 2.1. Materials

Sodium alginate (Protanal<sup>®</sup> LFR 5/60, high  $\alpha$ -L-guluronate (G) residues) was obtained from FMC Biopolymer (Sandvika, Norway). Polyallylamine (PAA; average molecular weight [MW] 15 kDa) was purchased from Polyscience Inc. (Warrington, PA, USA). Diethylene glycol, sodium periodate and dopamine hydrochloride (dopamine) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Dulbecco's phosphate-buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA

and antibiotic-antimycotic solution were purchased from Welgene Inc. (Daegu, Korea). All other materials were used without further purification.

### 2.2. Preparation of oxidized alginate

Oxidation of alginate was prepared following a previously described method, with slight modification.<sup>12</sup> Sodium alginate (3.0 g; 15.1 mmol uronate residues) was firstly dissolved in 70 mL deionized water (DDW). The alginate solution was mixed with 30 mL sodium periodate (4.53, 9.06, or 13.59 mmol) in DDW and incubated in a water bath at 50 °C for 4 h. The reaction was stopped by the addition of 10 mL ethylene glycol solution (10% v/v). Sodium chloride (8 g) was added to the solution, followed by precipitation in 600 mL ethanol. The supernatants were decanted, and the precipitates were re-dissolved in DDW. The solution was washed twice and purified by dialysis (MW cut-off, 12000-14000; Spectrum Laboratories Inc. Rancho Dominguez, CA) against DDW for 3 days. The solution was lyophilized for 3 days and kept in a desiccator until use. The analysis of OA composition was performed using Fourier transform-infrared (FTIR) spectrometer and <sup>1</sup>H NMR spectrometry (400 MHz Unity-Inova, Varian, USA). Aldehyde content of OA was measured by hydroxylamine hydrochloride titration.<sup>17,18</sup> The degree of oxidation (%) was calculated using the following equation:

$$\frac{(\text{Volume of mL NaOH} \times N \text{ NaOH}) \times 10^{-3} \text{ mol}}{\text{Weight of sample (g)} \times (\text{alginate MW})} = \frac{\text{mol of aldehyde}}{\text{mol of alginate}} \times 100 (\%)$$

The oxidized alginate was assigned to OA-30, OA-60 and OA-90 based on the estimated degree of oxidation (%).

### 2.3. Introduction of dopa to oxidized alginate

Dopa-OA was synthesized by Schiff base reaction between dopa and OA. Briefly, 1 mmol of OA-30 was dissolved in 10 mL DDW. Dopamine (0.2 mmol) in 10 mL DDW was added to the OA solution and incubated for 1 h at 37 °C. The solution was maintained at pH 5.0 (titrated with HCl). After the reaction, the product was purified by dialysis membrane (MW cut-off 12000-14000) against DDW at pH 5.0 for 3 days, and freeze-dried. The dopa content of Dopa-OA was measured by UV-vis spectrometry (Infinite 200 pro, Tecan, Männedorf, Switzerland). The degree of substitution (DS) with dopa was calculated from the absorbance at 280 nm using a standard dopamine solution.

### 2.4. Preparation of hydrogel glues

The hydrogel glues (OA glue and Dopa-OA glue) were prepared by mixing each oxidized alginate solution with PAA. Each 100 mg of OA-30, OA-60, and OA-90 was dissolved in 0.5 mL DDW under stirring at room temperature. 0.5 mL of PAA solution (15% w/v) slowly added to the OA solution. Dopa-OA glue was prepared by mixing Dopa-OA with PAA using the method described in the formation of OA glue. The gelation time of each glue was monitored by time-lapse video.

## 2.5. Measurement of rheological properties of hydrogel glues

Rheological properties of OA glue and Dopa-OA glue were measured on an advanced rheometric expansion system (ARES, Rheometric Scientific Inc., UK), using a cone-and-plate fixture with a 20 mm parallel plate. Time sweep measurement was performed to confirm the gelation time, immediately after mixing with an equal volume (0.1 mL) of OA and PAA (15%) solution on the plate. The frequency sweep was conducted by varying the angular frequency range was 0.6–120 rad/s.

## 2.6. Estimation of tissue adhesiveness

Tissue-adhesive strengths of hydrogel glues were evaluated by measuring the force required to detach them from a mucin disc using a universal testing machine (UTM, TopTac2000, Yeonjin Corporation, Korea).<sup>19,20</sup> The mucin discs were prepared using as previously described.<sup>21</sup> The discs were attached to the end of a cylindrical probe using double-sided adhesive tape. Each 100  $\mu$ L of OA and Dopa-OA with 100  $\mu$ L PAA (15% w/v) was placed on the holder of the UTM plate, which was then lowered until the mucin disc contacted the surface of the sample. A downward force of 100 g (0.981 N) was applied for 20 seconds, and the probe was moved upwards at a constant speed of 0.5 mm/s. The adhesion strength was determined as the maximum value achieved. All measurements were performed at least three times.

## 2.7. Estimation of hemostatic function on operative hepatic bleeding model

The *in vivo* hemostatic ability of the hydrogel glues was evaluated on operative hepatic bleeding model using male 6-week-old C57BL/6 mice weighing  $20 \pm 2$  g (Orient Bio, Seongnam, Korea).<sup>22,23</sup> Briefly, a mouse was fixed on a surgical cork board prior to making an abdominal incision. Serous fluid around the mouse liver was carefully removed and a pre-weighed filter paper on paraffin film was placed beneath the liver. The cork board was tilted and maintained at an angle of about 45°, and liver bleeding was induced by deep needle puncture (21 gauge). The test substance (50  $\mu$ L) was immediately applied to the bleeding site. The amount of blood absorbed on the filter paper was weighed after 3 min. All animal experiments were conducted in accordance with the approval and guidelines of the Institutional Animal Care and Use Committee at the Inha University.

## 2.8. Statistical analysis

Each experiment was repeated at least three times and the data were expressed as the mean  $\pm$  standard deviation. Statistical significance was assessed using a two-tailed Student's t-test, and a value of  $p < 0.05$  was considered statistically significant.

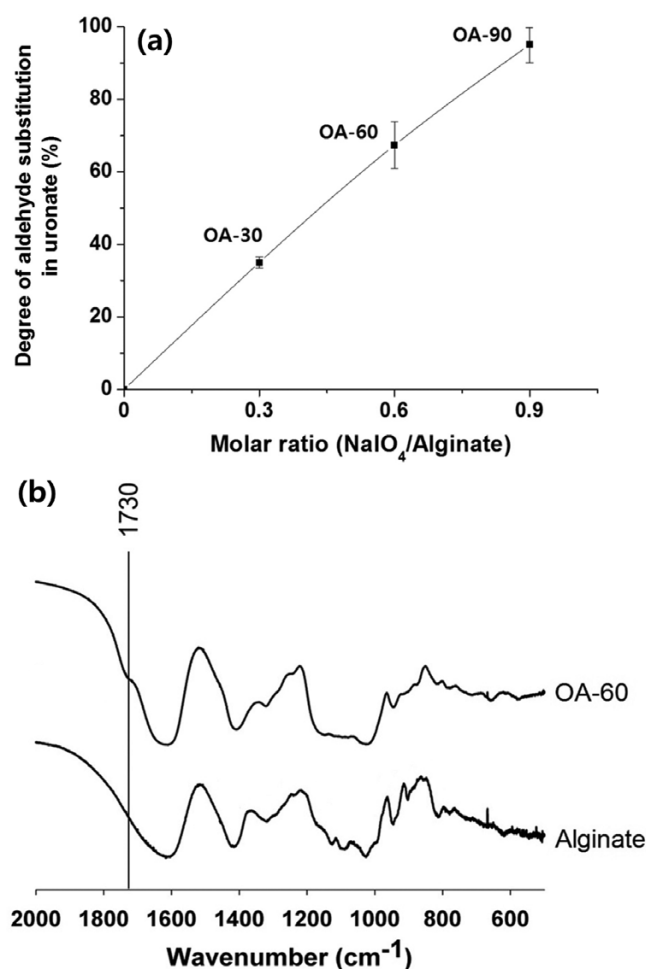
## 3. Results and discussion

### 3.1. Oxidation of alginate and gel formation property of hydrogel glue (OA glue)

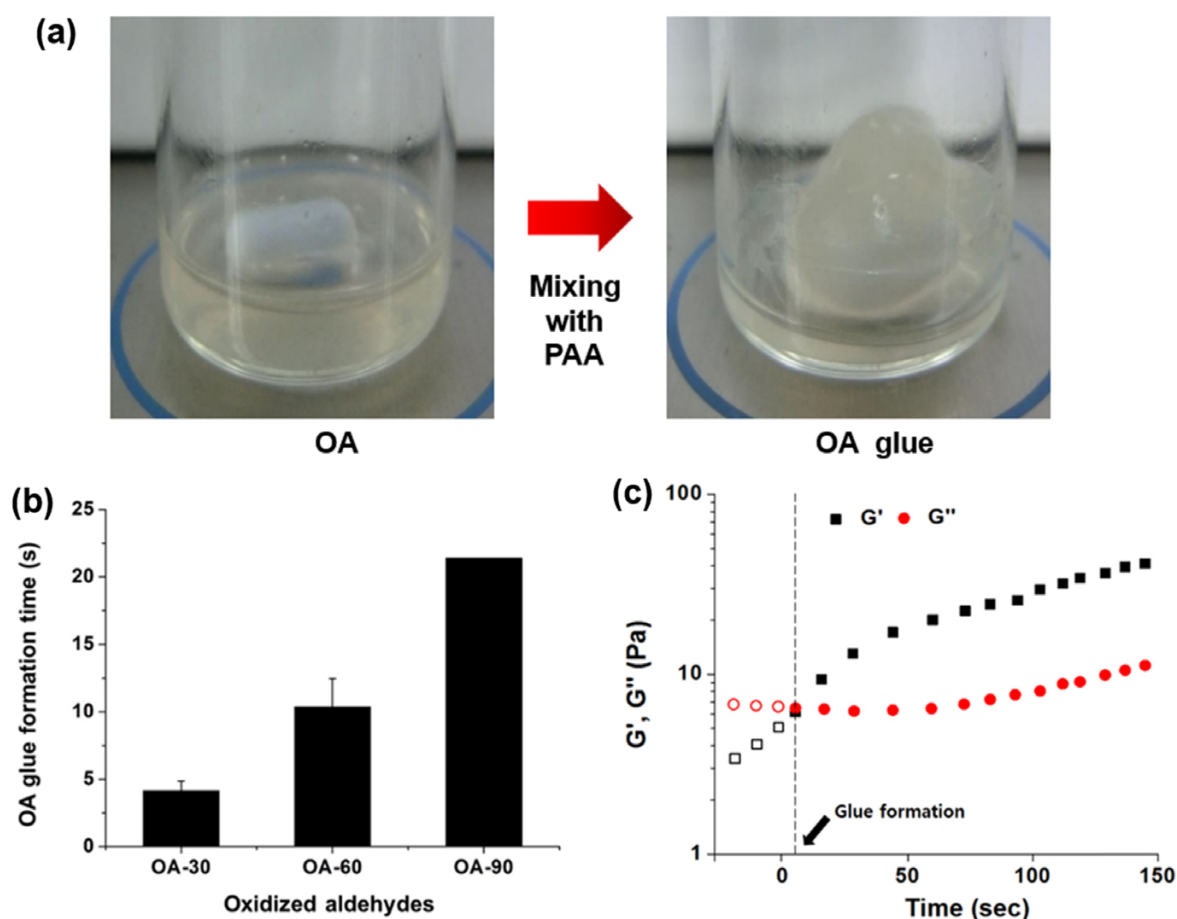
As described above, the oxidation of polysaccharide renders a

Schiff-base forming aldehyde which acts as main functional group of tissue adhesive glue. In this study, we observed how degree of oxidation affects the gelation property of OA hydrogel glue and the resulting adhesiveness. First, the application amount of the oxidizing reagent, sodium periodate, was varied by molar ratio of alginate sugar units and the degree of oxidation was determined by titration method. Figure 1(a) shows the molar content of aldehydes in alginate can be easily controlled by changing the applied amount of sodium periodate. Generation of aldehyde in alginate polymer was detected in FTIR and NMR spectra of the sodium alginate and the oxidized alginate (Figure 1(b) and Figure S1). FTIR spectrum of OA-60 proved a new characteristic peak at  $1730 \text{ cm}^{-1}$  (C=O), and NMR spectrum also shows two new signals at 5.3 and 5.5 ppm which correspond to the protons of hemiacetals formed from aldehyde and hydroxyl groups. These results indicated that sodium alginate was successfully oxidized by sodium periodate.

Generally, gelation is defined as phase transition of system from liquid-like state to solid-like state.<sup>24</sup> In our design, OA is immediately converts to hydrogel glue after mixing with PAA, and acts as a mechanical barrier for bleeding control. Three different OAs with different degree of oxidation (Figure 1(a)) was



**Figure 1.** (a) Oxidation (aldehyde formation) of alginate after treatment with sodium periodate by molar ratios (30, 60, and 90 mol% to mol of alginate units). (b) FTIR spectra of sodium alginate and oxidized alginate (OA-60).



**Figure 2.** (a) Instantaneous formation of OA glue after mixing oxidized alginate (OA-30) with PAA. (b) Glue formation time of each oxidized alginate by the degree of oxidation. (c) Time sweep rheological analysis of OA glue. Intersection point of the elastic ( $G'$ ) and viscous ( $G''$ ) modulus is designated to the glue formation (gel formation) time point.

introduced hydrogel formation study. Figure 2(a) shows OA transformed into hydrogel within a few seconds after mixing with PAA. Gelation time of OA-30, OA-60, and OA-90 was 4.1, 10.4, and 21.4 s, respectively (Figure 2(b)). The intersection of storage modulus ( $G'$ , ■) and loss modulus ( $G''$ , ●), observed after mixing the two polymers in time sweep rheology, typically represents hydrogel formation (Figure 2(c)). It was observed in 5 to 10 seconds after mixing of the two polymers. The frequency sweep analysis also showed typical property of hydrogel ( $G' > G''$ ) (Figure S3). In this study, we found OA-30 exhibited short gelation time and retained the initial mechanical properties of the alginate and was selected as the final component of hydrogel glue for subsequent experiments.

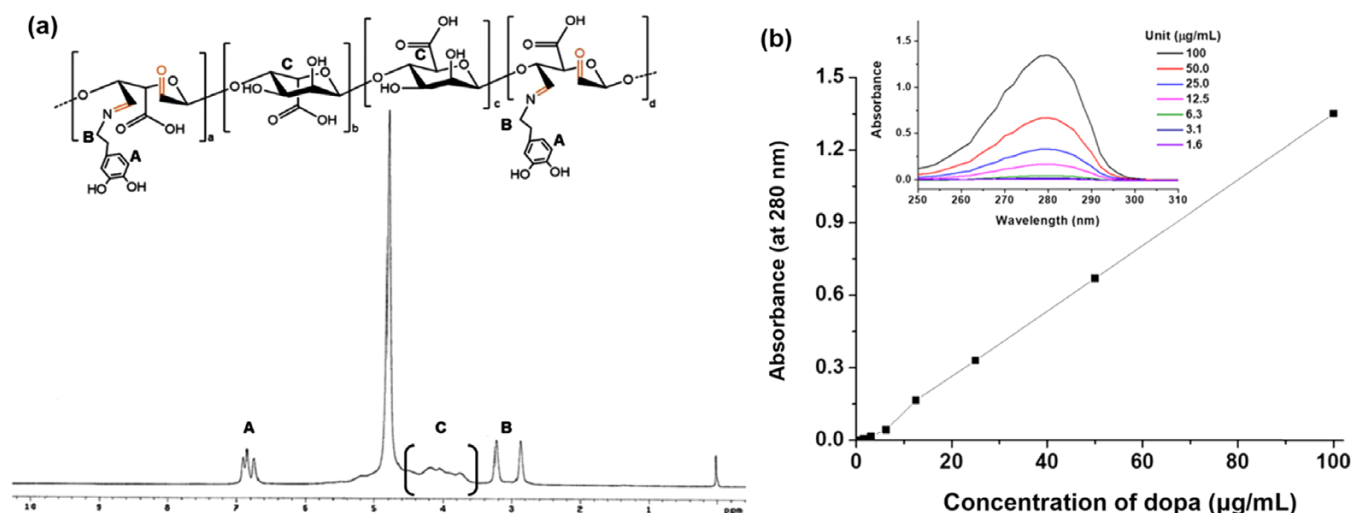
### 3.2. Introduction of dopa into OA and rheological properties of Dopa-OA glue

Schiff base formation determines the basic properties of hemostatic glue. In this study, we found OA glue become too rigid over time, easily detached from the tissue and might not assure the time-resistant hemostatic action. Therefore, we introduced dopa into OA to enhance the adhesiveness and flexibility of glue. Chemical structure of Dopa-OA was analyzed by  $^1\text{H}$  NMR spectrometry and UV-vis spectroscopy.  $^1\text{H}$  NMR (Figure 3(a)) shows

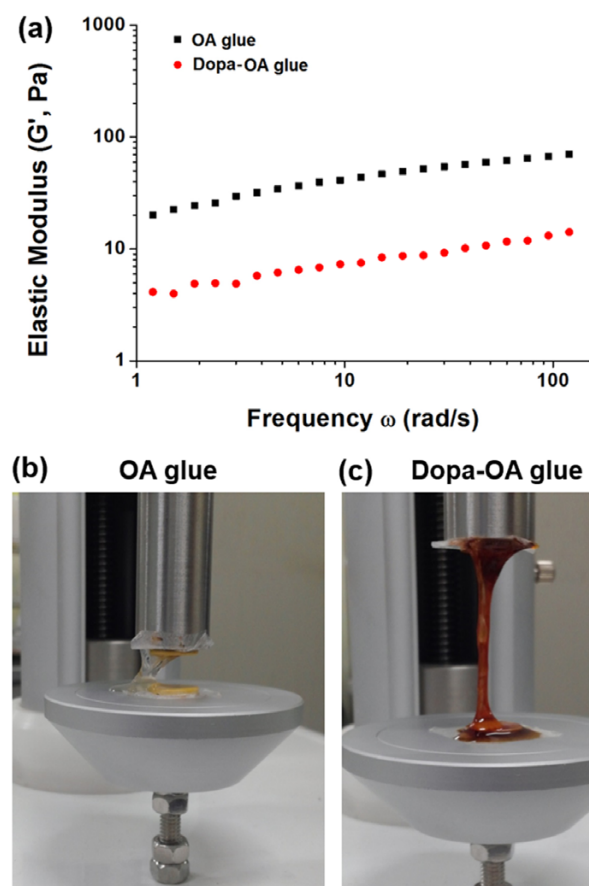
typical spectrum of Dopa-OA peaks; 2.9 and 3.1 ppm ( $\text{CH}_2$ , dopa), 6.67.0 ppm ( $^3\text{H}$ , aromatic ring proton, dopa), and 3.54.4 ppm ( $\text{CH}_2$ , partially oxidized alginate)).<sup>25</sup> Figure 3(b) displays a standard UV calibration curve for the determination of dopa concentrations in Dopa-OA polymer, and the estimated dopa content was around 10 mol.%. Dopa-OA glue was prepared by mixing Dopa-OA (20%, w/v solution) with PAA (15%, w/v solution). In the same manner as the OA glue, we observed the instantaneous formation of the hydrogel after mixing the two polymers ( $G' > G''$  data not shown). In a frequency sweep test,  $G'$  values of Dopa-OA glue reduced after introduction of dopa (Figure 4(a)). The elastic modulus of hydrogels showed not only the characteristics of solid-like material as expected after rapid formation of hydrogel but also rigidity of dopa-bound hydrogels (Dopa-OA glue) decreased.

### 3.3. Tissue adhesiveness of hydrogel glues

Tissue adhesion forces of OA glue and Dopa-OA glue were evaluated using tensile testing machine which is measured the maximum force required to detach the hydrogel from a mucin disc. The adhesive strength of OA glue and Dopa-OA glue was estimated  $655.1 \pm 34.4 \text{ gf/mm}^2$  and  $761.4 \pm 52.2 \text{ gf/mm}^2$ , respectively. Additionally, elongation power of OA glue and Dopa-OA



**Figure 3.** (a)  $^1\text{H}$  NMR spectra of Dopa-OA. (b) The calibration curve for dopa concentration using UV spectra at 280 nm.



**Figure 4.** Rheological properties of OA glue and Dopa-OA glue. After the introduction of dopa, the hydrogel glue showed the enhanced elasticity and elongation power. (a) Frequency sweep elastic ( $G'$ ) modulus of OA glue and Dopa-OA glue. (b) Tensile strength measurement of (b) OA glue and (c) Dopa-OA glue.

glue was observed in this study. OA glue detached from the mucin disk shortly when the head of UTM machine moved up. Meanwhile, Dopa-OA glue was unbroken and elongated during the test (Figure 4(b)). Dopa-OA glue displayed  $4.1 \pm 0.4$  folds higher elongation property than OA glue. These results suggested

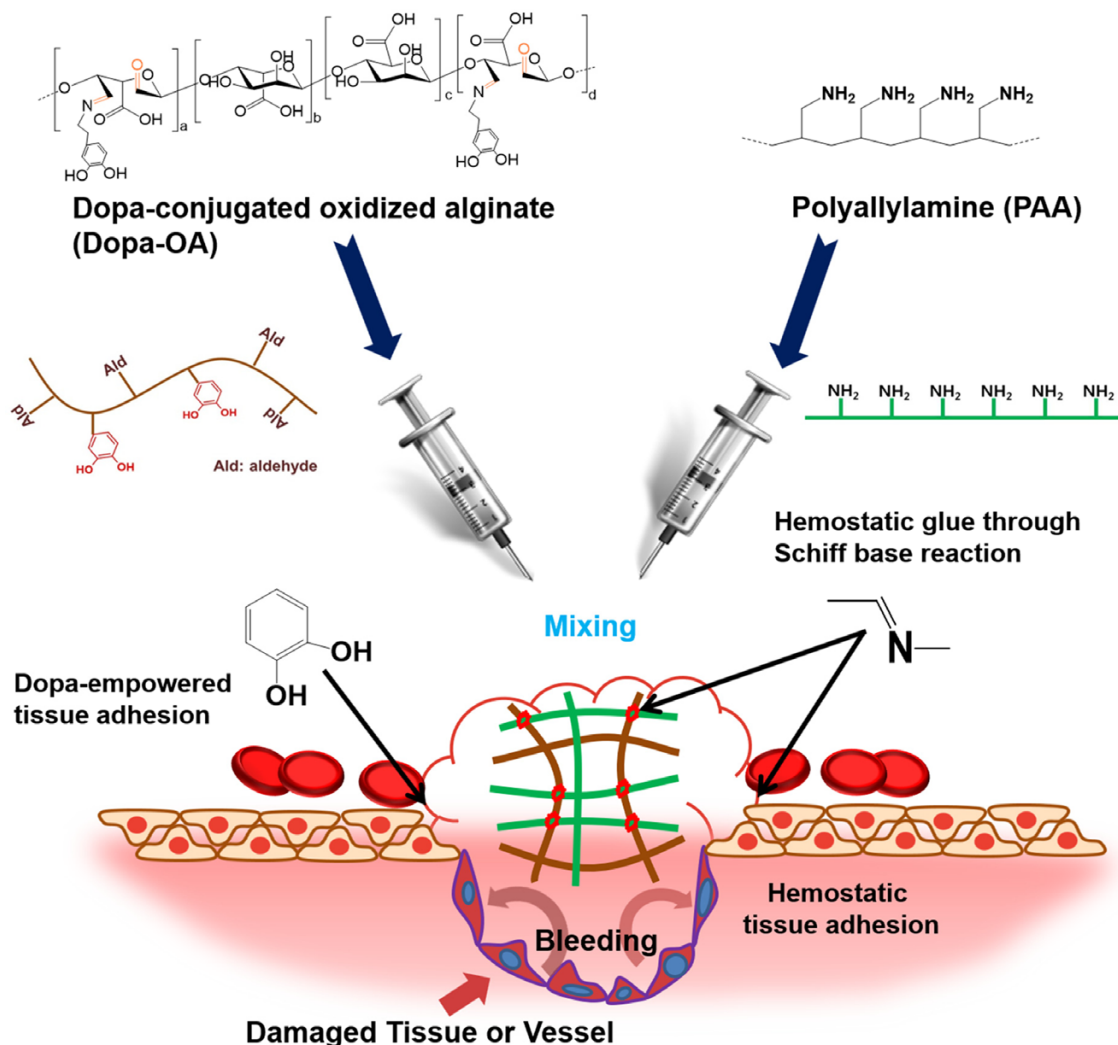
that introduction of dopa empowered adhesive property and elasticity of hydrogel glue.

#### 3.4. *In vivo* hemostatic action of hydrogel glue

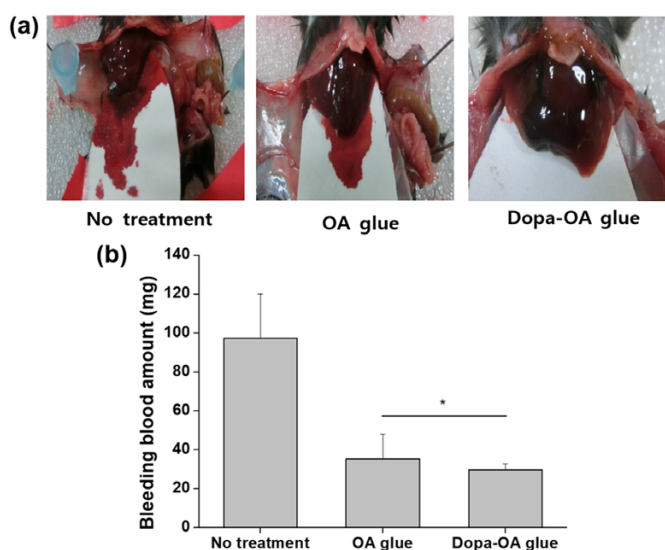
Scheme 1 showed the gelling formation and hemostatic procedures of Dopa-OA glue on wound tissue. Hemostatic function of hydrogel glues was evaluated in a C57BL/6 mouse model. Figure 5(a) showed the liver tissue images for 3 min after deep pricking with a needle, with or without glue application. Figure 5(b) shows the total amount of blood released. The average amount of blood lost in the absence of hydrogel was 97.4 mg. The average amounts of blood in mice treated with OA glue and Dopa-OA glue were 35.2 and 29.7 mg, respectively ( $n=3$ ,  $p<0.05$ ). The applied hydrogel systems successfully stopped the bleeding in this model by attaching to the wound site and undergoing a phase change from solution to tissue adhesive glue. As observed in music adhesion test, OA glue was more easily detached from the bleeding site by exudate body fluid, as compared with Dopa-OA glue. Dopa-OA glue showed much higher stickiness and elasticity than OA glue. These results indicated that Dopa-OA glue was a good candidate for the rapid treatment of hemorrhage.

#### 3.5. Discussion

The present study employed alginate for the design of hemostatic hydrogel glue because of its biocompatibility and rapid gel-formation property.<sup>8,12</sup> To achieve the hemostatic function, Schiff base forming aldehyde and mussel-inspired functional dopa group were co-introduced into the alginate (Scheme 1). First, Schiff base forming aldehyde was rendered by the oxidation of alginate using sodium periodate (Figure 1) and then dopa was introduced *via* Schiff base forming between the amine residue of dopamine and the aldehyde of OA. Practically, OA alone could not act as a hemostatic adhesive since the gel formation properties of alginate disappeared after the oxidation and requires addition of intra-structuring polyamines. So, OA glue was constructed *via* intra-Schiff base network between aldehyde of OA and amine of PAA (Figure 2(a) and Scheme 1).



**Scheme 1.** A schematic diagram of hemostatic glue formation after mixing dopa-conjugated oxidized alginate (Dopa-OA) with polyallylamine (PAA). The oxidized alginate generates Schiff base with PAA and tissue amine, and instantaneously forms hydrogel glue. The introduction of dopa enhances the elasticity of hydrogel glue and empowers the hemostatic glue property.



**Figure 5.** (a) Hemostatic action of OA glue and Dopa-OA glue on mouse liver surgical hemorrhage model. (b) Total bleeding amount of each glue treatment ( $n=3$ ,  $*p<0.05$ ), Blood was collected for 3 min after the hepatic puncture.

In this study, we selected PAA as a Schiff-base forming polymer. Schiff bases is induced between an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine. Primary amine is the best candidate for this reaction, and PAA has properties such as a high density of amine groups, a good water solubility and low toxicity after cross-linked form. We also assume long aliphatic chain of PAA allows the stronger binding capacity with target gene as well as powerful gel strength after the reaction with OA.

Schiff base reaction between the aldehydes of OA and the amine groups of tissue proteins allows the formation of stiff mechanical barrier for hemorrhage control. But adhesiveness of OA glue decreases and eventually detached from the wound tissue over time; we, therefore, introduced mussel-inspired dopa into OA and compared the physical properties and hemostatic abilities with OA glue (Figure 4 and 5). The elasticity (storage modulus,  $G'$ ) of Dopa-OA glue was lower than that of OA glue, and this result suggested that Dopa-OA glue possessed much better feasibility for application at the bleeding site than OA glue. Additionally, tissue adhesion force of Dopa-OA glue ( $761.4\pm 52.2$

gf/mm<sup>2</sup>) was significantly higher than that of OA glue (655.1±34.4 gf/mm<sup>2</sup>). Figure 5 also showed that the elongation of Dopa-OA glue (*i.e.*, stickiness) was significantly increased (around 4 folds) after introducing dopa in the hydrogel system.

To evaluate the hemostatic action of the adhesive systems on hemorrhage, the amount of blood loss was compared after applying various hydrogels. Both OA glue and Dopa-OA glue produced successful hemostatic effects in a mouse liver hemostasis model. However, we observed OA glue detached from the tissue over time because of its decrease in adhesiveness. Our observation indicated that Dopa-OA glue showed superior hemostatic function due to tissue conjugation *via* the Schiff base reaction and dopa quinone reaction. Introduction of dopa allowed an elastic and mussel-pad like adhesive property. Therefore, Dopa-OA glue could provide a more effective hemostatic tissue-adhesive than OA glue.

#### 4. Conclusion

In this study, dopa-empowered and Schiff base forming alginate hydrogel glue (Dopa-OA glue) was developed for hemostatic glue. The hemostatic glue based on Dopa-OA showed suitable physical features, including good rheological properties, and tissue adhesion. In addition, Dopa-OA glue effectively arrested bleeding on the hepatic bleeding animal model. Therefore, Dopa-OA glue can provide a novel tissue adhesive for the treatment of hemorrhage in surgical or trauma settings.

**Supporting information:** Information is available regarding the characterization of Dopa-OA glue and synthetic scheme of Dopa-OA glue. The materials are available *via* the Internet at <http://www.springer.com/13233>.

#### References

- (1) J. F. Kelly, A. E. Ritenour, D. F. McLaughlin, K. A. Bagg, A. N. Apodaca, C. T. Mallak, L. Pearse, M. M. Lawnick, H. R. Champion, and J. B. Holcomb, *J. Trauma*, **64**, S21 (2008).
- (2) L. Montanaro, C. R. Arciola, E. Cenni, G. Ciapetti, F. Savioli, F. Filippini, and L. A. Barsanti, *Biomaterials*, **22**, 59 (2001).
- (3) P. Ferreira, R. Pereira, J. F. J. Coelho, A. F. M. Silva, and M. H. Gil, *Int. J. Biol. Macromol.*, **40**, 144 (2007).
- (4) D. M. Toriumi, K. O'Grady, D. Desai, and A. Bagal, *Plast. Reconstr. Surg.*, **102**, 2209 (1998).
- (5) S. Canonico, *Acta Biomed.*, **74**, Supple 2:21 (2003).
- (6) K. H. Siedentop, J. J. Park, A. N. Shah, T. K. Bhattacharyya, and K. M. O'Grady, *Am. J. Otolaryngol.*, **22**, 230 (2001).
- (7) R. Bitton and H. Bianco-Peled, *Macromol. Biosci.*, **8**, 393 (2008).
- (8) O. Jeon, J. E. Samorezov, and E. Alsberg, *Acta Biomater.*, **10**, 47 (2014).
- (9) A. B. Lansdown and M. J. Payne, *J. R. Coll. Surg. Edinb.*, **39**, 284 (1994).
- (10) A. Sank, M. Chi, T. Shima, R. Reich, and G. R. Martin, *Surgery*, **106**, 1141 (1989).
- (11) C. M. Gao, M. Z. Liu, J. Chen, and X. Zhang, *Polym. Degrad. Stab.*, **94**, 1405 (2009).
- (12) K. H. Bouhadir, K. Y. Lee, E. Alsberg, K. L. Damm, K. W. Anderson, and D. J. Mooney, *Biotechnol. Prog.*, **17**, 945 (2001).
- (13) B. Balakrishnan and A. Jayakrishnan, *Biomaterials*, **26**, 3941 (2005).
- (14) J. H. Ryu, Y. Lee, W. H. Kong, T. G. Kim, T. G. Park, and H. Lee, *Biomacromolecules*, **12**, 2653 (2011).
- (15) S. H. Baik, J. H. Kim, H. H. Cho, S. N. Park, Y. S. Kim, and H. Suh, *J. Surg. Res.*, **164**, E221 (2010).
- (16) Y. Lee, H. J. Chung, S. Yeo, C. H. Ahn, H. Lee, P. B. Messersmith, and T. G. Park, *Soft Matter*, **6**, 977 (2010).
- (17) H. Zhao and N. D. Heindel, *Pharm. Res.*, **8**, 400 (1991).
- (18) X. M. Mo, H. Iwata, S. Matsuda, and Y. Ikada, *J. Biomater. Sci. Polym. Ed.*, **11**, 341 (2000).
- (19) D. S. Jones, C. R. Irwin, A. D. Woolfson, J. Djokic, and V. Adams, *J. Pharm. Sci.*, **88**, 592 (1999).
- (20) D. S. Jones, A. D. Woolfson, A. F. Brown, W. A. Coulter, C. McClelland, and C. R. Irwin, *J. Control. Release*, **67**, 357 (2000).
- (21) D. S. Jones, M. L. Bruschi, O. de Freitas, M. P. D. Gremiao, E. H. G. Lara, and G. P. Andrews, *Int. J. Pharm.*, **372**, 49 (2009).
- (22) Y. Murakami, M. Yokoyama, H. Nishida, Y. Tomizawa, and H. Kurosawa, *Colloids Surf. B: Biointerfaces*, **65**, 186 (2008).
- (23) Y. Murakami, M. Yokoyama, H. Nishida, Y. Tomizawa, and H. Kurosawa, *J. Biomed. Mater. Res. Part B: Appl. Biomater.*, **91B**, 102 (2009).
- (24) J. D. Ferry, E. R. Fitzgerald, L. D. Grand, and M. L. Williams, *Ind. Eng. Chem.*, **44**, 703 (1952).
- (25) S. Hong, K. Yang, B. Kang, C. Lee, I. T. Song, E. Byun, K. I. Park, S. W. Cho, and H. Lee, *Adv. Funct. Mater.*, **23**, 1774 (2013).